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Warming intensify CO_2 flux and nutrient release from algal wrack subsidies on sandy beaches

Running head: Effect of warming on algal wrack cycling

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Abstract

Algal wrack subsidies underpin most of the food web structure of exposed sandy beaches, and are responsible of important biogeochemical processes that link marine and terrestrial ecosystems. The response in decomposition of algal wrack deposits to global warming has not been studied in ocean exposed sandy beaches to date. With this aim, passive open top chambers (OTCs) were used to increase soil temperature within the range predicted by the

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IPCC for western Europe (between 0.5 and 1.5 ºC), following the hypothesis that the biogeochemical processing of macroalgal wrack subsidies would accelerate in response to temperature increase. The effect of temperature manipulation on 3 target substrates: fresh and aged macroalgae, and bare sand, was tested. Results indicated that a small warming (< 0.5 ºC) affected the wrack decomposition process through traceable increases in soil respiration through CO_2 flux, inorganic nutrients within the interstitial environment (N and P), sediment organic contents measured through the amount of proteins and microbial pool through the total soil DNA. The different responses of soil variables in the studied substrates indicated that the decomposition stage of stranded macroalgae influences the biogeochemical processing of organic matter in sandy beaches. Thus, CO_2 fluxes, releases of organic and inorganic nutrients and microbial activity intensify in aged wrack deposits. Our results predict that expected global warming will increase the release of inorganic nutrients to the coastal ocean by 30 % for the N (21 Gg y^{-1}) and 5.9 % for P (14 Gg y^{-1}); that increase for the flow of C to the atmosphere as CO_2, was estimated in 8.2 % (523 Gg y^{-1}). This study confirms the key role of sandy beaches in recycling ocean derived organic matter, highlighting their sensitivity to a changing scenario of global warming that predicts significant increases in temperature over the next few decades.

Key words: Wrack decay, Open top chambers, Nutrients, Organic enrichment, CO_2, DNA, Global warming.

Introduction

Accumulation of macroalgae and plant debris strandings, also known as wrack, is a common event in the intertidal sedimentary coastal rim, worldwide. These external inputs of allochthonous biomass derive from detached primary producers of the neighbouring ecosystems, such as seaweeds from rocky shores, and vascular plants from sea grasses and salt marsh areas (Griffiths *et al.,* 1983; Colombini and Chelazzi, 2003; Dugan *et al.,* 2003; Orr *et al.,* 2005). The ecological influence of algal wrack is of relevance where a highly productive coastal environment (such as subtidal rocky shores) interfaces with and exports materials to the less productive sandy beaches, normally devoid of aquatic macrophytes (McLachlan & Brown, 2006). As subsidized environments, open coast sandy beaches are responsible for recycling variable amounts of heterogeneous allochthonous organic matter, which underpins most of the beach food web, both in the subtidal (Crawley *et al.,* 2006), intertidal (Soares *et al.,* 1997; Dugan *et al.,* 2003; among others) or supratidal beach (Dugan *et al.,* 2003; Lastra *et al.,* 2008; Duarte *et al.,* 2014; Ruiz-Delgado *et al.* 2017). Wrack subsidies strongly influence ecological features and functioning of the receiver ecosystem, boosting biodiversity and abundance of primary and secondary consumers (e.g. Ince *et al.,* 2007; Olabarria *et al.,* 2007; Crawley *et al.,* 2009; Spiller *et al.,* 2010; Wilson & Wolkovich, 2011).

Algal wrack processing is the degradative sequence along which organic matter accumulated in the sediment from algal subsidies is recycled into inorganic nutrients and CO_2 through mineralization and respiration (Anschutz *et al.,* 2009). Consumption is an initial and paramount pathway within the biological processing of wrack supplies to sandy shores; for example, stranded macroalgae in temperate latitudes sustain large populations of supratidal crustaceans and insects, which are frequently the most abundant taxa, comprising up to 90% of the beach macrofauna (Stenton-Dozey & Grifiths 1983; Dugan *et al.,* 2003, Duarte *et al.*

2014, Ruiz-Delgado *et al.* 2015). Following deposition, macroalgal wrack starts to be fragmented, decomposed and mineralized by the beach macrofauna, meiofauna and bacteria (Koop *et al.,* 1982; Mews *et al.,* 2006; Coupland & McDonald, 2008; Salathe & Riera, 2012). Wrack processing at final stages seems to be dominated by bacterial activity (Hubas *et al.,* 2007), resulting in CO_2 respiration and the release of inorganic nutrients (Buchsbaum *et al.,* 1991; Chapin *et al.,* 2002; García-Robledo *et al.,* 2008; Hardison *et al.,* 2010). The efficiency of algal wrack in supporting the basic metabolism of the associated food web can be measured through the quantification of the CO_2 fluxes and the inorganic N pool, as an index of the soil community respiration and mineralization (Coupland *et al.,* 2007; McCulley *et al.,* 2004; Lützow & Kögel-Knabner, 2009; Berfurg & Friberg, 2012). The conversion of organic matter into inorganic nutrients is considered a key ecosystem service that connects algal wrack decay with primary producers and consumers (Catenazzi & Donnelly, 2007). Wrack deposits behave as a source of labile C and N, which enhances microbial biomass, sediment respiration and N mineralization (McCulley *et al.,* 2004; Lomstein *et al.,* 2006). Since beaches function as sea-land ecotone, the beach runoff can enhance the productivity of coastal waters through discharge with nitrate, nitrite, ammonia and phosphorous (Maier & Pregnall, 1990; Brooks *et al.,* 2008; Dugan *et al.,* 2011; Barreiro *et al.,* 2011). Raised metabolism in beach cast can last for several weeks or months, depending on wrack characteristics and environmental conditions (Barreiro *et al.,* 2011; Rossi *et al.,* 2011).

Physicochemical features of the organic source seem to be an influencing factor in controlling wrack processing within the interstitial environment (Hubas *et al.,* 2007); for example, different species (Duarte *et al.,* 2010) and between tissues variability within a single species of stranded macroalgae (Duarte *et al.,* 2011) could prompt distinctive biogeochemical footprint, by being processed at different rates throughout the consumption pathway. Similarly, variability in nutritional value and/or habitat features provided by the physical

structure of the wrack, enclose distinct colonizing faunal assemblages in search for food and shelter (Buschmann, 1990; Rodil *et al.,* 2008; Duarte *et al.,* 2010). Within-species variability in the fate of wrack deposits also occurs when differences exist in the life history of the algal clump after detachment from the original substrate; e.g., decomposed macroalgae are consumed at a faster rate than when fresh (Lastra *et al.,* 2015), which supports the hypothesis that the biogeochemistry of the algal wrack processing differs depending on the aging state of stranded material.

Increase in temperature is a prominent aspect of the global climate change that is projected to accompany modifications in biogeochemistry and metabolism of the coastal ecosystems worldwide; functional shifts linked to climate change will include changes in productivity, nutrients cycling and food web dynamics, which could have a direct impact on ecosystem services from coastal habitats. Changes in soil respiration and carbon and nitrogen cycling are expected to occur as a consequence of the temperature increase (Norby *et al.,* 1997; Liboriussen *et al.,* 2011; IPCC 2014 Synthesis Report); hence, it is predicted that warming will raise beach metabolism by speeding decomposition rate of algal deposits, the nutrient cycling and the CO_2 emission (Coupland *et al.,* 2007; Lützow & Kögel-Knabner, 2009). Besides, warming will accelerate the processing of wrack deposits in sandy beaches by speeding the feeding rate of primary consumers, such as Talitrid amphipods and insects (Lastra *et al.,* 2015; Duarte *et al.,* 2010), with unpredicted cascading effect on trophic web structure (Dugan *et al.,* 2003). Other stressors related with the global warming such as acidification and CO_2 availability, is expected will have harmful effects on algal wrack consumption by affecting consumers behaviour or algal palatability (Poore *et al.,* 2013; Duarte *et al.,* 2016).

There is a lack of scientific contributions setting out to understand the biogeochemical processes involved in the decomposition of algal wrack deposits in a scenario of global warming. The most optimistic models associated with climate change predict an increase in temperature of *ca*. 0.5 to 1.5 ºC over the next few decades in western Europe, with an average rate of 0.1 ºC per decade (IPCC 2014 Synthesis Report). Thus, the principal issue in this study was to clarify potential consequences of global warming in the fate and processing of algal wrack subsidies in sandy beaches. Specifically, the effect of increasing temperature was assessed on: 1) the rate at which macroalgal decay occurs once stranded on the beach; 2) the amount of organic and inorganic nutrients release drained toward the sediment through the decomposition process; and 3) the CO_2 fluxes associated with decomposition, as a proxy of the metabolic rate and biogeochemical activity. To fulfil these objectives, a field experiment was performed in one intermediate exposed sandy beach on the NW coast of Spain. Passive open top warming chambers (hereafter OTCs) were used to manipulate air and soil temperature by mimicking a greenhouse effect (Marion *et al.,* 1997) on patches of macroalgae with different life histories (*i.e.* fresh vs. aged). OTCs have been extensively used in climate change and ecosystem functioning studies in terrestrial ecology (e.g. Sharkhuu *et al.,* 2013 and references herein). Our experiment in field conditions represents an improvement in the design of manipulative field experiments aimed at understanding the biogeochemical links between beach metabolism, wrack subsidies and global warming at ecosystem level.

Materials and Methods

Study site

Experiments were conducted on Ladeira beach (42° 34' 36'' N, 9° 3' 20'' W), an exposed sandy beach about 2 km long and 130 m wide (low spring tide), located in the NW coast of Spain. This beach is backed by a large, active dune system and is characterized by a mesotidal range of *ca*. 3.5 m during spring tides. It harbours a diverse and variable macrophyte wrack supply, usually distributed in heterogeneous patches stranded along the drift line and in the upper beach, mainly composed of brown algaes *Saccorhiza polyschides*, *Sargassum muticum* and *Cystoseira* spp (Barreiro *et al.,* 2011). Weather conditions correspond with a mild oceanic climate, with mean daily temperatures ranging between 7 and 24 ºC throughout the year (www.meteogalicia.es).

Baseline in wrack supply to the supratidal beach was estimated along a monthly sampling field work, carried out in Ladeira beach between March 2012 and March 2013; Wrack coverage was measured according to the method proposed by Dugan *et al.* (2003). Overlapped beach (m) was transformed into dry weight biomass (g) according to the linear the regression: Wrack biomass (g m^{-1} DW) = 108.3 x Coverture (m) + 20.41, obtained by Lastra *et al.* (2014). The baseline results on algal wrack coverture showed that 59 % (± 22) of the algal supplies received by the beach strand above the drift line, covering an average of 12.5 % (±15) of the upper beach surface. This means that an average of 16.7 g m^{-2} dry weight of wrack deposits occupy the upper beach surface year round.

Pilot study

A pilot study was performed in advance of the experiment setup in order to calibrate the warming effect of the OTCs; thus, 1 data logger was buried in the sand at 5 cm depth at each of 3 OTCs installed in bare sand areas; to control for temperature changes, 1 data logger was similarly buried at each of 3 in bare sand sites without OTCs. After 7 days, the mean temperature in the OTCs was 25.3 ºC (±0.52), whereas that in the controls was 24.2 ºC (±0.17). Temperature differences obtained were within the target range, and compatible with the temperature increase predicted by the IPCC for the next decades in the studied area (0.5 to 1.5 ºC) (IPCC 2014 Synthesis Report).

Field study

We designed a manipulative field experiment to test for temperature-induced changes in macroalgal wrack decay over time, resembling what occurs with stranded material between two consecutive spring tides. The blade-shaped brown macroalgae *Saccorhiza polyschides* (Lightfoot) Batters 1902 (hereafter, *Saccorhiza*) was used as the target species, for being the dominant stranded macroalgae during the study period and over the year cycle (Barreiro *et al.*, 2011). Detached macroalgae from rocky bottoms can drift during variable periods of time in a broad range of states of decomposition before reaching the shore (Crawley & Hyndes, 2007). Thus, as a proxy of what naturally occurs in exposed beaches, two different decomposition stages of *Saccorhiza* were tested in the experiments: 1) fresh fronds collected within the 24 h prior to the experiment setup; 2) aged fronds that were obtained by aging fresh *Saccorhiza* in the water column. Aged material was prepared similarly to Dethier *et al.*, (2012) by holding 1 mm mesh bags loaded with 10 kg of fresh algae at 5 m depth during one week (n= 3). All the initial algal material was collected live from the neighbouring rocky

shore of the Toralla Marine Science Station of the University of Vigo (42º 12,07' 05'' N; 8º 48,03' 16'' W). To homogenise the composition within each type of algal wrack, fronds were cut in fragments of 20 cm long (aprox.) and all the material was carefully mixed before experiment setup.

The experiment started on 24th June 2014 and lasted until 14th July. Twelve cone-shaped open top passive warming chambers (OTC) made of compacted polycarbonate (light transmission > 90%; Makrolon® GP clear 099, Bayer Sheet Europe), measuring 1.1 m diameter at the bottom, 0.65 m at the top, 0.6 m in height and 1 mm thick (Figure 1S_SuppInfo), were used to investigate the effect of temperature on biogeochemical processes associated with wrack decay.

Experimental algal plots consisted of 4 circular patches (90 cm ∅) of fresh algal fronds and 4 of aged fronds, 3 Kg each, that were randomly distributed parallel to the shoreline at *ca*. +0.5 m in height above the drift line, as the upper limit of stranded material transportation by the swash at spring high tide (Figure 1S_SuppInfo). Algal amount used in the experiment mimicked the density of algal wrack deposits in the studied area during the summer period (Barreiro *et al.*, 2011; Gómez *et al.*, 2013). To control for the biogeochemical effect of the algal patches, 4 bare sand positions were also randomly included in the experimental stretch. Next, 12 OTC´s were positioned on top of each of the algal patches, both fresh and aged, and on the bare sand sites. Positions provided with OTCs were referred to as warmed treatments. The lower edge of the OTCs were lifted 10 cm above ground to allow for sediment movement to occur naturally, and to avoid screen effect of the chambers against the aeolian transportation of sand, that could have create artificial differences between treatments and control plots. To track for the temperature, one data logger (± 0.2 ºC accuracy; Onset Computer Corporation, Bourne, MA, USA) was buried at 5 cm deep in the sand, according to

McCulley *et al.*, (2004), at the center of each of 2 plots (out of 4, chosen at random) of any treatment and control assay; temperatures were measured at synchronized intervals of 20 minutes throughout the 20 days of the experiment time; with this, mean temperature and max-min range were calculated. Degree-days were estimated using the area under the curve of the daily average temperature (Jaki & Wolfsegger, 2009). Because of a technical failure, only one series of temperature was recorded on fresh algae under OTCs.

To control for the warming effect obtained within the OTCs, algal patches of fresh and aged *Saccorhiza* (n= 4, 3 kg each) and bare sand, but without OTCs, were randomly included along the experiment stretch; these were referred to as un-warmed controls. All plots were placed 5 m apart each other, and their positions were marked with aluminum sticks inserted into the sand.

To obtain values at the start of the experiment (t_0), 4 algal patches of fresh, 4 aged *Saccorhiza*, 3 kg each and 90 cm ∅, as well as 4 bare sand positions, were set in the surroundings and at the same tidal position of the experimental plots. CO_2 fluxes in algae and bare sand plots ($\mu M\ m^{-2}\ s^{-1}$) were measured by pushing vertically into the patches a 20 cm diameter accumulation chamber with infrared gas analyser (IRGA) of a WS1101 West Systems Portable Soil CO_2 Flux Meter (Parkinson 1981); algae directly covered by the accumulation chamber were carefully removed and transported to the laboratory for further analyses of algal dry weight and total organic C, N and P. Next, 3 corers 3 cm diameter were pushed into the sediment till 5 cm depth (as done by Canion *et al.*, 2014) in the area that was directly underneath the algae to analyse sediment granulometry, moisture, proteins and inorganic nutrients (NH_4^+, NO_3^-, NO_2^- and PO_4^{-3}). Sediment corers were also collected to quantify total DNA, as an estimator of the microbial biomass within the sediment at the start of the experiment. All the samples were immediately frozen at -20 ºC until processing. Similarly, one 10 cm diameter corer to a depth of 10 cm was collected underneath any

fresh/aged algal patch and bare sand (n= 4 each) to evaluate initial macroinfauna; these samples were sieved with 1 mm mesh and the remnant was fixed with 70% ethanol and stored for species composition and abundance.

On days 3, 7 and 20 of the experiment, each patch of fresh or aged algae and bare sand, either warmed or un-warmed, was sampled as above. CO_2 data were obtained between 12:00 and 14:00 at every sampling date; hence it was assumed that temperatures were maximum within the circadian cycle. To calibrate for the effect of the dial cycle of temperature in the CO_2 emission, a parallel experiment was conducted where fluxes were measured in patches of 500 g of *Saccorhiza* deployed above the drift line, at different times: 7:30 a.m., 10:30 a.m., 13:00 and 15:30, thus covering the full range of daily temperatures. Temperatures were minimum between 7:30 and 8:30, and reaching maximum after 14:00 at any date of the experiment (www.meteogalicia.es). Therefore, a correction factor of 0.77 was obtained to standardize for the CO_2 emission at the mean temperature along the daily cycle. Due to logistic constrains, this study was conducted in a beach (Abra: 42º 09' 11'' N; 8º 49' 53'' W) in which algal wrack characteristics, sediment features (Barreiro et al. 2011) and weather conditions were similar to those existing in the study site where the OTC experiment was conducted.

Meteorological information along the trials was supplied by the nearest neighbouring meteorological station located within the beach-dune system, 2.2 Km from the study site (www.meteogalicia.es).

Laboratory analyses

Sediment mean grain size was estimated by dry sieving (Folk, 1980), and sediment moisture was determined by weight loss after drying at 60 ºC until constant weight (Giere *et al.,* 1988). Total organic C and N of fresh and aged algal tissue at initial time t_0 were measured with a LECO model CNS 2000; P was quantified by inductively coupled plasma optical emission spectrometry.

The sediment samples (10 g aprox.) were shaken in the dark for 2 hours in 25 ml, 0.01 mol/l KCl solution for inorganic N extraction; the solution was then centrifuged at 4600 rpm 10 minutes and the filtered extract (quantitative filter paper of 2–4 µm) was stored at -20 ºC until processing (Barreiro *et al.,* 2013). Nutrients were quantified by continuous flow analysis in a Bran Luebbe Nutrient Analyzer AA3. Ammonium was measured fluorometrically at 460 nm following excitation at 370 nm following the method of Kerouel & Aminot (1997); the samples were reacted with ophthalaldehyde (OPA) at 75 °C in the presence of borate buffer and sodium sulphite, to form a fluorescent species in a quantity that is proportional to the ammonium concentration. NO_2- and NO_3- were analysed via the diazo-reaction based on the methods of Armstrong *et al.* (1967) and Grasshoff *et al.* (1983). This automated procedure involves reduction of NO_2- to NO_3- by a copper-cadmium reductor column; the NO_2- then reacts with sulphanilamide and N-1-napthylethyleneidiamine dihydrochloride under acidic conditions; the concentration was determined colorimetrically at 550 nm. Nitrification products hereafter will be referred as the sum of NO_2-+NO_3- (as in Brooks *et al.,* 2008), as concentrations were highly correlated (R^2= 0.95, P< 0.0001). Total inorganic N (hereafter TIN) was calculated as the sum of NH_4^+, NO_2- an NO_3-. Phosphate analyses were based on the colorimetric method of Murphy & Riley (1962), in which a blue colour is formed by the

reaction of orthophosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH of 1; the reduced blue phospho-molybdenum complex is determined colorimetrically at 880 nm.

These analyses were carried out in the ‘‘Elemental Analysis Unit’’ of the University of Vigo. Data units for all the inorganic nutrients were in µM per g of dry sediment samples; nutrients on bare sand were also expressed per square metre (top 5 cm) by using averaged values per g of sediment throughout the experiment (n= 12) multiplied by the sample size (7.07 cm^2, 44.1 g ± 4.4). To allow for comparison with studies where nutrient concentration were measured in pore water, data on µM g^{-1} of dry sand were transformed to µM, following Brooks *et al.* (2008), assuming 40% of saturation volume (Barreiro *et al.,* 2013; Charbonniere *et al.,* 2013) and a dilution factor of 1.77 (25 ml) accountable to the extraction method.

Contents in total proteins were gauged as a proxy of the edible organic matter received by the beach system (Fabiano *et al.,* 1995; Lastra *et al.,* 2016). A more conventional method as dry-combustion in a CHN-LECO was not used in the calculation of sedimentary organic matter cause is based in the assumption that all carbon combusted is associated to organic matter, what means that the carbonated sediments will produce a strong interference when they are depleted in organic matter, as commonly occur in exposed sandy beaches. Total proteins were determined using the method of Lowry & Rosebrough (1951) modified by Markwell *et al.* (1978). Concentrations were referred as bovine serum albumin equivalents. Three g of sediment were used for the analyses of each sample. For each test, blanks were made using the same sediments previously treated in a muffle furnace (500 ºC 6 h). Protein concentrations were converted into carbon equivalents and total N using 0.85 (Simon & Azam, 1989) and 0.18 (Shuuluka *et al.,* 2013) conversion factors, respectively.

The specific extraction kit "Quant-iT dsDNA Assay High Sensitivity" followed by Qubic Fluorometric Quantitation was used to quantify total DNA in the top-5 cm sediment, as a proxy of the bacterial and fungal communities contributing for the organic matter decomposition (Agnelli *et al.*, 2004). Calculations were expressed as $ng \cdot g^{-1}$ of sediment.

Data treatment

Temperature data for each treatment and control did not accomplished with normality requirements; therefore, as measurements from data-loggers were synchronized, the non-parametric Wilcoxon test for paired data was used to compare soil temperature obtained in the OTC treatments for each type of substrate (fresh algae, aged algae and bare sand) against the two corresponding controls. The change in water contents over time for fresh and aged *Saccorhiza*, both in controls and OTC treatments, served as a measure of intra-specific variability in wrack decomposition. Decomposition expressed as the water contents of algal patches was evaluated with a single exponential decay model (following Jędrzejczak, 2002): $Wt = W_0^{e-kt}$ where, W_t is the weight loss (%) of a given sample remaining after time (t). W_0 is the initial dry weight (g) of a given sample (*i.e.* t_0); k is the decay coefficient (day^{-1}) and t is the time (days). The decay coefficient (k) was used to compare decomposition rates between different treatments at each time interval.

All the physicochemical variables were time scaled. Changes in the algal moisture, CO_2 fluxes, proteins, nutrients (Nitrites-Nitrates, Ammonia and Phosphates), total DNA and the wrack and sediment macrofauna (*i.e.* total abundance and species richness) over time, were analysed using a three-way ANOVA; decomposition state (fresh/aged algae and bare sand controls, 3 levels) and treatment (OTC´s *vs.* controls, 2 levels) were considered as orthogonal fixed factors, and time (3 levels) was considered a random factor. Student−Newman−Keul's

test (SNK) was used for *a posteriori* comparisons of factors ($p < 0.05$). Contrasting across treatments/controls within substrates were performed through One-way ANOVA with *a posteriori* multiple comparisons Tukey test. The D'Agostino & Pearson test was used to examine for normality. The homogeneity of variances was evaluated with Cochran's test.

For the total soil respiration over the 20 days of experiment, the trapezoidal rule was used to calculate the area under the curve, following McCulley *et al.* (2004); since CO_2 emissions were estimated in $\mu M\ m^{-2}\ s^{-1}$, calculations were based on a sequence of 1728000 seconds. Total organic carbon to nitrogen ratio (C/N) of the initial tissues was calculated as an index of edibility of algal substrates used in the trials. Coupling between TIN and CO_2 flux was examined through regression analyses.

Increase in $N\text{-}NH_4^+$, $N\text{-}NO_3^- + NO_2^-$, PO_4^{-3}, proteins and DNA concentration in the surface sediment was calculated through the bulk values per g^{-1} of sand subtracted by the corresponding values in bare sand, either treatments or controls. Recycling rates of N and P were obtained by dividing the maximum soil concentrations in $N\text{-}NH_4^+$, $N\text{-}NO_3\text{-} + NO_2\text{-}$ and PO_4^{-3} over the 20 days of experiment by the total N and P contents in the averaged biomass of algal patches at initial time (48.85 g ± 18.8 dry weight). The mean residence time of algal C was obtained by dividing the averaged bulk of organic C in the algal patches, by the total soil respiration over the 20 days of experiment, corrected by those rates measured in the corresponding bare sand treatment or control. As data from IRGA flux meter CO_2 are devised per m^{-2}, averaged algal biomass collected underneath the 20 cm Ø chamber (48.85 g dry weight) was extrapolated to m^2 (=1555 g).

Results

Mean temperatures in the sediment underneath the OTCs at the 3 target substrates studied were warmer than those measured in the control plots, and differences were significant: Wilcoxon test for paired data, $P< 0.001$ in all cases, except for one of the fresh algae plots with warm induced treatment, that was $P= 0.07$. Increases were as follows: +0.03 ºC for the fresh algae (0.14%), +0.33 ºC in aged algae (1.56%) and +0.13 ºC in bare sand (0.59%). The effect seems to be more conspicuous when calculations are formulated in degree-days (Table 1), which results in increases of 1.64%, 1.23% and 1.45% for the fresh and aged patches and bare sand, respectively.

Average sand moisture in plots with and without algae were 2.0 % (±1.7) and 1.44 % (± 1.44), respectively, without statistical differences between warming-induced plots and controls. The interaction between treatment and algal substrates was significant, which means that the three substrates behave unequally; *e.g.* sand moisture under fresh algae was higher in control plots than under the chambers (ANOVA: Treatment x Substrate interaction, $F_{2,54}= 8.04$; $P< 0.001$; SNK test: $P< 0.01$); however, sediment under aged wrack was dryer under controls than in OTCs patches (ANOVA: Treatment x Substrate interaction, $F_{2, 54}= 8.04$; $P< 0.001$; SNK test $P< 0.01$). Moisture in bare sand under OTCs and in the control plots was always similar (SNK test $P> 0.05$). Maximum sediment moisture was measured at day 9 of the experiment (t_2), reproducing the pluviosity and water balance reported from the nearby meteo station. Negative water balance occurred in the field throughout the experiment, except for days 3, 5 and 10 (www.meteogalicia.es); thus, water loss was the prevailing state, hence depleting moisture of the sediment and wrack patches.

Water contents of fresh and aged *Saccorhiza* at the start of the experiment were 90.4 ± 0.2% and 91.3 ± 0.3 %, respectively. Decomposition rate (*i.e.* K, Day $^{-1}$) indicated that maximum weight loss by dehydration was observed at day 3 (t_1) for all the treatments and controls (Figure 2S_SuppInfo), with values ranging between 0.48 (fresh algae under OTC) to 0.72 (aged algae in controls). Desiccation continued at a rate of 0.15 % day^{-1} during the final stages of the experiment. Water contents of the algal tissues under OTCs were higher than those measured in the control plots (ANOVA: Treatment, $F_{1, 48} = 7.36$, $P< 0.01$). Changes in mean dry weight (DW, %) of the fresh algae occur according to the formulas: $DW= 1.33\ e^{-0.24\ t}$ and $DW= 0.74\ e^{-0.18\ t}$ in the controls and warm induced plots, respectively; whereas those for the aged patches were $DW= 1.1\ e^{-0.16\ t}$ and $DW= 0.81\ e^{-0.16\ t}$ for the controls and OTC plots, respectively (Figure 2S_SuppInfo). Difference in moisture between algal tissues of fresh vs. aged *Saccorhiza* was not significant (ANOVA: Substrate, $F_{1, 48} = 1.52$, $P= 0.22$), neither the desiccation trend over time (ANOVA: Treatment x Substrate x Time interaction, $F_{3, 48} = 0.3$, $P= 0.82$).

Total inorganic N (TIN) in the sediment underneath the OTCs was higher than that in the control sites, although differences were statistically significant only when comparing treatment *vs*. control of aged macroalgae (Figure 3S_SuppInfo) (ANOVA: Treatment x Substrate interaction, $F_{2, 54} = 0.98$, $P= 0.38$, SNK test $P< 0.05$). The TIN measured in the sediment below the aged *Saccorhiza* was higher than that under fresh algaes, and this occurs both when comparing OTC plots or controls (SNK test $P< 0.05$ in both cases). Total N in the tissues of the fresh and aged *Saccorhiza* at the start of the experiment were 1.85 % ± 0.06 and 2.22 % ± 0.2, respectively, which is within the range measured in similar species of Phaeophyceae (Gevaert *et al.,* 2001). Results indicate that 0.3 % (55 µg g^{-1} of alga) of the total N initially confined in the fresh algae in the control plots, and 0.45 % (83 µg g^{-1} of alga) of that in warmed sites were mineralized as TIN and transferred to the sediment throughout

the experiment. Aged algae leached 0.79 % (176 µg g^{-1} of alga) and 0.99 % (220 µg g^{-1} of alga) of their total N as TIN to the sediment, for the control and OTC plots, respectively. Maximum increases of TIN in the sediment were always observed at the end of the experiment time, t_3 (20 days), except for fresh algae in OTC plots that was at t_2 (7 days).

Warming through OTCs increased in 13.2 % the average amount of NH_4^+ in plots with fresh macroalgae, and in 26 % of that in aged patches, although these increases were not statistically significant (Fig. 1a). Averaged amount in NH_4^+ in the sediment of the bare sand throughout the experiment was lower than that under algal patches, although differences were only significant when comparing with warm induced patches of fresh algae or aged deposits (see Fig. 1a for details). Averaged NH_4+ concentration in samples of bare sand was 3.2 ± 1.5 µM g-1 of sand in control plots; that for the heated sites was 2.5 ± 1.3 µM g^{-1} of sand; these values correspond with a interstitial water concentration of 56 ± 26 µM and 44 ± 23 µM. Warming also triggered an increase of 24 % and 35 % in NO_2-+NO_3- in sand under fresh and aged macroalgae, respectively (Fig. 1b).

Mean NO_2-+NO_3- in bare sand was 7 ± 3.3 µM and 8.3 ± 4.6 µM for control and OTC plots respectively, which is equivalent to 124 ± 58 µM and 146 ± 81 µM in the interstitial water. There were no differences in NO_2-+NO_3- between sand samples from bare sand areas and those under patches of fresh algae (Fig. 1b); NO_2-+NO_3- below aged algae roughly duplicate the values obtained in bare sand, albeit differences were only significant when comparing aged algae under OTCs and control patches of bare sand (One way ANOVA, $F_{5, 66}$ = 3.57; Tukey HSD test, $P< 0.05$). Variability in time showed that increases in soil NH_4^+ peaked at the 9th day for all the substrates and treatments (Fig. 2a). Maximum increases in NO_2- + NO_3- in the sediment occurred at the end of the experiment (*i.e.* 20 days), except under fresh algae in warm-induced plots (Fig. 2b).

Warming seems to deplete leaching of PO_4^{-3} in fresh algal plots, whereas it increased in those of aged macroalgae (ANOVA: Treatment x Substrate interaction, $F_{2,54} = 5.06$, $P<0.01$, SNK test: $P< 0.01$), and has no effect on bare sand plots (Fig. 1c, Fig. 2c). Total P within the tissues of fresh and aged *Saccorhiza* at initial time were 2.71 % and 1.85 %, respectively; after 20 days of experiment, 2.29 % (619 µM g^{-1} of alga) of the total PO_4^{-3} of the fresh algae in the control plots, and 0.78 % (619 µg g^{-1} of alga) of that under OTCs were transferred to the sediment. In aged algae, 2.3 % (425 µM g^{-1} of alga) of the PO_4^{-3} in the control samples, and 5.1 % (942 µM g^{-1} of alga) of that in warmed sites, was released to the substrate.

Mean value of sediment organic N under control patches of fresh algae was 20.9 µM g^{-1} (± 4.9), whereas that under OTCs was 22.2 µM g^{-1} (± 4) (Figure 4S_SuppInfo). In aged algae, organic N under control patches was 22 µM g^{-1} (± 4.6), with that in warm-induced plots reaching to 37.9 µM g^{-1} (± 12.7). Values in bare sand were 15.2 µM g^{-1} (± 2.5) and 18.5 µM g^{-1} (± 6.8), what means 0.95 M m^{-2} (± 0.4) and 1.16 M m^{-2} (± 0.5) in control and OTC sites, respectively. Sediment organic N in bare sand plots were equivalent to 457 µM (± 192) and 555 µM (± 247) in interstitial water, for the control and OTC plots, respectively. Averaged amount in organic N in the sediment throughout the experiment was not statistically different across treatments and substrates (Figure 4S_SuppInfo), except for the patches of aged algae under OTCs when compared with control patches of bare sand (One way ANOVA, $F_{5, 66} = 2.31$; $P= 0.053$ Tukey HSD test, $P< 0.05$).

Fresh algae, either control or warmed, released as much as 2 % of their total N initially confined in their tissues, as organic N toward the sediment, which represents 377 µM and 372 µM per g of alga for control and OTC plots, respectively. Aged algae leached 2 % and 4.7 % of their total N as organic N to the sediment, what in this cases means 448 µM g^{-1} of algae and 1053 µM g^{-1} of algae for the control and OTC plots, respectively.

Organic N and TIN in the sediment increased under patches of algae until day 9; then, organic N decreased whilst TIN increased, what indicates an active mineralization. This trend was particularly conspicuous in the sediment under aged algae, in which TIN after 20 days of experiment accounted for 62 % and 61 % of the total N in the controls and warmed plots, respectively (Figure 3S_SuppInfo). For the controls plots of aged algae, time variability in TIN involves mineralization rates of 2.15 µM d^{-1} g^{-1} of sediment between days 3 and 9, and 0.5 µM d^{-1} g^{-1} between days 9 and 20. Calculation in warm-induced conditions results in mineralization rates of 1.12 µM d^{-1} g^{-1} between days 3 and 9, and 1.16 µM d^{-1} g^{-1} between days 9 and 20.

Initial total organic carbon contents in the tissues of fresh and aged *Saccorhiza* were 25.6 % and 30.8 % respectively, without differences in C:N ratio (14.39 in both cases; t-test= 0.004, P= 0.99). Mean sediment organic C over the 20 days of experiment in control plots of fresh algae was 98 µM g^{-1} (± 34), whereas that under warming-induced plots was 104 µM g^{-1} (± 35). Mean organic C in sediment was 102 µM g^{-1} (± 35) under control patches of aged algae, with those in warm-induced plots reaching to 167 µM g^{-1} (± 91.1). Values in bare sand were 76 µM g^{-1} (± 16) and 84 (± 39), what means 5.1 M m^{-2} (± 2.1) and 6.2 M m^{-2} (± 2.7) in control and OTC sites, respectively. There were no statistical differences in organic contents in the sediment across treatments and controls (One way ANOVA, $F_{5, 66} = 1.1$; P= 0.37).

Warm-induced plots emitted larger volumes of CO_2 than the controls, but differences were not significant for any of the substrates assayed (Fig. 3). CO_2 fluxes from aged algae were larger than those from fresh algae, and differences were significant (One way ANOVA, $F_{5, 90} = 7.16$, P< 0.001, Tukey HSD test, P< 0.05 in all the cases).

Patches of algae, either fresh or aged, released larger volume of CO_2 than bare sand plots (Fig. 3), although only values from aged algae were significantly different (Tukey HSD test,

P< 0.01 in all the cases). Decomposed algal patches released more than 50 % of CO_2 within the first 3 days; the decline in water contents of the algal tissues was concurrent with the abrupt drop in CO_2 emission after the third day. Averaged CO_2 emission from fresh algal plots increased 7 % when heated under the OTCs (Fig. 3). When measuring in plots with aged algae, warming caused an increase of 8.2 % in the CO_2 flux toward the atmosphere. The results indicate that 0.6 % of the organic C stored in the fresh algaes in control plots (*i.e.* 1600 μM C g^{-1} of algae) and 0.65 % of that in warmed plots (*i.e.* 1724 μM C g^{-1} of algae) was respired as CO_2 along the 20 days of experiment. Warming increased in 17 % the flux of organic C of aged algae, from 3070 μM C g^{-1} of algae (1.5 % of the stored C) in control plots, to 3594 μM C g^{-1} (1.81 % of the stored C) under OTCs. Bare sand from treatment and control patches released less CO_2 than plots covered with algae (Fig. 3), albeit differences were only significant when comparing with aged algae (Fig. 3) (One way ANOVA: $F_{5, 90}$= 7.16; P< 0.001, SNK test P< 0.01 in all cases). Warming treatment also triggered a 13% increase in CO_2 flux of the bare sand beach surface, which means that raised from 3.13 to 3.54 mM m^{-2} day^{-1} on average throughout the experiment time.

CO_2 flux and TIN were related according to the formula CO_2= 0.1 + 0.19 log TIN, R^2= 0.86; P< 0.001 (Fig. 4).

Total DNA in the sediment increased over time during the decomposition process (Fig. 5). There were not significant differences in DNA when comparing sediment from OTCs and control plots (One way ANOVA: $F_{3, 44}$= 0.35, P= 0.78, Tukey test P> 0.05 in all cases). Averaged DNA concentration in the sediment under fresh patches of alga was 6.2 ng DNA g^{-1} (± 4.3), whereas that under OTCs was 6.9 ng g^{-1} (± 5.8). The mean estimate of DNA under aged wrack in control patches was 8.5 ng g^{-1} of sediment (± 6.6), while that under OTCs was 7.3 ng g^{-1} (± 4). Mean contents in DNA in the sediment below fresh algae were 2.43 ng DNA g^{-1} (± 2.46), and 4 ng DNA g^{-1} (± 4.9) in warm induced plots and in the controls, respectively.

Those values for aged algae were 4.1 ng DNA g^{-1} (± 4.2) and 4.1 ng DNA g^{-1} (± 3.6) in OTC plots and in the controls, respectively.

The DNA in the sediment under patches made of fresh algae increased constantly over time (Fig. 5a), while DNA in the sediment under aged patches peaked at day 9 (t_2), with no further significant change (Fig. 5b). Averaged soil DNA in control bare sand areas was 3.1 ng g^{-1} of sediment (± 2.8), which, in warm-induced plots, was 2.2 ng g^{-1} (± 0.8). There were significant differences between sediment DNA underneath fresh and aged macroalgae and bare sand, (ANOVA: Substrate, $F_{2,54}$= 14.13; P< 0.001**)**, as follows: aged algae = fresh algae < bare sand (Figure 5S_SuppInfo).

There were not statistical differences in beach macrofauna across treatments and substrates. The low number of individuals collected (n= 31 in total) prevented to obtain any conclusion on the effect of macrofaunal assemblages on the algal wrack processing. The coleopteran *Phaleria cadaverina* (Frabicius, 1792) (n= 10) and the Oniscidea isopod *Tylos europaeus* Arcangeli, 1938 (n= 10) were the most abundant species collected (n= 10).

Discussion

The results of this study indicate that open top chambers are suitable for conducting manipulative experiments addressed to evaluate the effect of short scale temperature increase on wrack metabolism in the un-moistured sediment of the supratidal beach. Sediment temperatures in plots provided with OTCs were warmer than those in control plots; the mean temperatures and degree days inside and outside of the OTCs were consistent and aligned with the experiment goals. Minimum daily temperatures seem to be buffered under the chambers, *i.e.* the mean of the lowest daily temperatures in the sediment were obtained in

control sites. The mechanism underlying this trend is unknown, but however, we hypothesize that OTCs, besides providing green house effect during day-light, can reduce heat loses during low temperature night-hours (Bokhorst *et al.*, 2013). Short scale variability in temperatures along the study site can be related with local variability in the wind conditions, what could have affected differently to the different plots along the transect where the experiment was conducted. Similarly, the lower rim of the OTCs was 10 cm uplifted to allow for aeolian transportation of sand, and to avoid screen effect of the chambers; thus, differential, albeit natural, sediment accretion/erosion on top of the plots could lead to an unpredicted effect on the temperatures underneath the sediment.

Despite the short differences in sediment temperatures between warm-induced plots and controls (see Table 1), the response of the studied variables was significant in a number of cases. Warming accelerated soil metabolism by speeding the bacterial activity (Spilmont *et al.*, 2005). Contribution of beach macrofauna to decomposition process of algal wrack through fragmentation and consumption (Hammann & Zimmer, 2014) was negligible, taking into account the small number of herbivore individuals collected in any substrate, either treatment or control. Besides temperature, the decaying stage of the algal patches determined the biogeochemical fate of the wrack biomass, meaning that aged algae delivered larger decomposition outputs, compared with those from fresh algal patches. The transformation of organic matter into inorganic nutrient that fertilize the coastal waters is considered an important ecosystem service of exposed sandy beaches (Schlacher *et al.*, 2013, 2015); wrack processing implements a substantial feed back that connects high productive macrophyte based environments, such as rocky shores, with the low productive neighbouring sandy shores (Barreiro *et al.*, 2013). The degradative pathway for mineralizing organic matter and the consequent nutrient release to the interstitial environment was enhanced in the OTC sites. Nitrification of NH_4^+ to NO_2^-+NO_3^- occurred rapidly under OTCs and in aged algae (less than

3 days). However, nitrification in patches of fresh *Saccorhiza* at ambience temperature seems to be initially buffered, as only NH_4+ and no NO_2-+NO_3- were measured after 3 days of decay. Ammonia could directly leach to the sediment from intracellular pools, since macroalgae accumulate this nutrient in large concentrations, (Corzo & Niell, 1991; Boyer & Fong 2005; García Robledo *et al.*, 2008). There was no pluviosity recorded during the first 3 days of the experiment, and therefore, nutrients percolated into the sediment column by gravity without dilution. Peaks of ammonia were measured after 9 days, then decreased while NO_2-+NO_3- increased. The mineralization process with increasing TIN while decreasing organic N was conspicuous in aged algae in warm environment; the effect of the initial state of the aged algal patches in activating the inorganic N and P output can indicate that breakable cell structure of decomposed tissues and the rapid loss in deterrent molecules, such as phenolic compounds, facilitated a rapid use of the decomposed algal tissues by beach microbiota (Buchsbaum *et al.*, 1991). This pattern has already been observed on beach macrofaunal herbivores, which feed preferentially on algae that have undergone several days of previous decay (Lastra *et al.*, 2015).

Few studies have been published to date providing detailed information on the beach metabolism and the nutrients dynamics of the dry sediments from the supratidal beach. Wrack deposits stranded at the higher tidal level in spring tides remain during several weeks or months on the sand surface (Rodil *et al.*, 2008), thus being exposed to meteorology, including desiccation, overheating, freezing, radiation, etc (Orr *et al.*, 2005). Moisture either in the organic deposits or in the substrate, determines the rates at which bacterial mineralization and nitrification operates (McCulley *et al.*, 2004, 2007; Hollister *et al.*, 2006). The results showed that metabolic rates throughout the decay process measured as CO_2 emission decline with the decrease in the humidity of the deposits. Sand moisture during the experiment was always below 3%, whereas water contents of the algal biomass dropped from

90 % to 20 % after day 3 for all the treatments and controls; hence, reduced decay rates of algal tissues obtained after t_1 were related with an algal breakdown buffered by desiccation.

The amount of algal C and N liberated in our experiment was small (ca. 3 % and 1.8% respectively) compared with what occurs in saturated sediments, where up to 6.4% and 35% of algal C and N respectively enrich the sediment after a time span similar to that in our assay (Hardison *et al.,* 2010). Likewise, our results were below the values obtained when decay occurs in the water column, where more than 40% of macroalgae biomass is released to the environment (Duarte & Cebrian, 1996; Kristensen, 1994; Wada *et al.,* 2007). When comparing with NO_2-+NO_3- in the pore-water, our data indicates that concentration in the upper beach bare sand (124 µM) was higher than those obtained in the intertidal beach by Brooks *et al.,* (2008), who reported yearly average values of 3.9 µM; values were also higher than those in pore water measured by Charbonniere *et al.,* (2013) and Barreiro *et al.,* (2013) in two monitoring studies of the intertidal zone in beaches on the French and Spanish Atlantic coast (17.4 µM and 14.1 µM, respectively). In contrast, our results were below values obtained in beaches that receive massive macrophytes subsidies along the year, as those along the Californian coast (Dugan *et al.,* 2011), where intertidal pore water range between 6 and above 10000 µM of TIN.

The long residence time of algal wrack after stranding during spring high tides, the rapid desiccation of algal tissues as well as the low moisture of superficial sediment, could be responsible for an accretion of recalcitrant algal debris in the upper beach. As a result, a significant amount of nutrients are stored in the supratidal zone, where only spring high tides, storms and pluviosity could contribute to accelerate soil metabolism. In the tidally affected beach, a short turnover rate determines the low organic contents of the beach sands (Anschutz *et al.,* 2009). Bacterial mineralization of the organic matter from wrack supplies occurs at accelerated rates, with inorganic N being used rapidly by primary producers (preferring NO_3-

) and bacteria (preferring NH_4+) (Brooks *et al.*, 2008; García Robledo *et al.*, 2008). There is a lack of information about the proportion of fresh vs. aged state of wrack subsidies received by exposed sandy beaches. Studies on turnover rate are scarce, but some calculations demonstrate replacement rates as high as 56 ± 18% (Lastra *et al.*, 2014) of wrack supplies within a 24 hours cycle; thus, a proportion of 50/50 % in fresh vs. aged material reaching the beach can be assumed. According to the calculations of the baseline study, the average algal wrack biomass of 16.7 g m^{-2} (dry weight) on the upper beach zone year round, means that 0.024 M m^{-2} of N should have been present in the sediment at the time when the experiment started. Data on bare sand control samples pointed out that the upper beach dry zone accumulates 0.15 M m^{-2} of inorganic N and 0.95 M m^{-2} of organic N, which represents around 4500 % above the total N expected. Similarly, averaged values of P obtained in bare sand areas during the experiment (0.95 M m^{-2}) were above values expected year round (0.12 M m^{-2}).

Averaged organic C from bare sand patches was 5.7 M m^{-2}, what is above values expected according to the calculations of the year-round study (0.38 M m^{-2}). As started above, we hypothesize that C and N from previous strandings remains in the spot as a consequence of a weak physical and biological fractioning of the wrack biomass, long periods out of tidal innundation and desiccation. These factors influence on the chances of mineralization by bacterial assemblages and the possibilities of nutrients to percolate into the sediment (by pluviosity, tidal inundation, sea spray, etc).

The augmented nitrification and CO_2 emission throughout the wrack decaying process in warm-induced environment could be linked with a boost in microbial activity, rather than an increment in microbial biomass, according to the non-significant difference in DNA contents between the sediment within the OTCs and those in control plots. The lack of statistical differences in the TIN contents between sediment underneath fresh algal patches and that

from bare sand plots without wrack, contrast with the clear rise in CO_2 emission through patches supplied with algae, either fresh or aged.

Carbon is respired as CO_2 through bacterial metabolism, which is mainly dependent on the algal wrack occurrence and humidity (Visser and Parkinson 1992; McCulley *et al.,* 2004; Hollister *et al.,* 2006; Li *et al.,* 2015). Soil respiration measured as CO_2 fluxes may represent a good proxy for ecosystem carbon cycling rates (Sharkuu *et al.,* 2013). Our data indicated that CO_2 flux increased under warming conditions, as well as on the different substrates, as follows: aged algae > fresh algae > bare sand. The relationship between CO_2 flux and TIN in the sediment highlights the coupling between microbial metabolism and mineralization. Why aged material intensify soil respiration was not tested, but we hypothesize that the partial degradation of algal tissues facilitated leaching of edible C and N to the sediment, thus supplying organic substrate that enhanced the microbial activity (McCulley *et al.* 2004; Hardison *et al.,* 2010). The C:N relationship of both fresh and aged *Saccorhiza* were similar, which means that other factors may be facilitating the decomposition process. Phenolic contents can drop drastically in algal tissues once senescence or decomposition starts (Lastra *et al.,* 2015), which is expected to facilitate wrack decay and consumption through loss of deterrent traits. Moreover, a surplus in bacteria coating the algal fronds can be responsible for the large respiration flow measured at initial time in aged *Saccorhiza*.

The low number of consumer macrofauna, associated with wrack patches, in particular herbivores, is a common feature of the beach studied (Lastra, unpublished data); therefore, physical fragmentation and microbial activity are major protagonists of the fragmentation, decaying and mineralization of the algal biomass (Porri *et al.,* 2011; Salathe & Riera, 2012).

Thus, the lack of macrofaunal contribution in this experiment delayed the decomposition process of the algal wrack biomass, what means that our results in beach metabolism could be enhanced in similar beaches with higher density of wrack-associated consumers of algal wrack subsidies, as Talitrid amphipods and Oniscidea Isopods.

Although the warming produced by the OTCs in this experiment setup was within the range of the most optimistic predictions of the IPCC report (2014) for western Europe, the consequence in terms of C and N cycling from wrack subsidies to sandy beaches are of relevance. Macroalgal production worldwide is estimated to be 1521 Tg C y^{-1} (Duarte & Cebrian, 1996), which means around 5390 Tg algal biomass (DW). There is not much information on the amount of macroalgal productivity that reaches the shore, but studies suggest that between 18 % and 44.4 % of the macroalgal production strand in the shoreline within 10 to 20 days after detachment from the original substrate (Hobday, 2000; Krause-Jensen & Duarte, 2016). Assuming an averaged supply of 31.2 % of the total production to the surrounding sedimentary shores, means that 1681 Tg y^{-1} of algae strand along the beaches worldwide, which can be translated into 34 Tg y^{-1} of N and 476 Tg y^{-1} of C (Gevaert *et al.*, 2001). According to our baseline data, 59 % of the stranding wrack is stored on the supratidal beach, which means that, on a global scale, 991 Tg y^{-1} of algae remain within an environment where decomposition process is buffered, compared with what occurs in saturated sediments or in the water column for an equivalent period of time. Increases in temperature within the range predicted by the IPCC (0.5 to 1.5 ºC over the next decades) will accelerate the beach metabolism during wrack decomposition in the supratidal beach; as a consequence, ulterior release of inorganic nutrients to the coastal water could increase by 30 % for the TIN (21 Gg y^{-1}) and 5.9 % for P (14 Gg y^{-1}). That increase for the flow of C to the atmosphere as CO_2, can be estimated in 8.2 % (523 Gg y^{-1}).

The uppermost layers of the dry sand in the supratidal beach zone, seem to be featured by a buffered decomposition rate of the allochthonous wrack subsidies. Low moisture and infrequent inundation events determine long residence time of nutrients and reduced C cycling through the interstitial environment. The upper beach thus acts as a storage compartment, compared with the active biogeochemical hot-spot that characterizes the permeable intertidal sediments. Uprising temperature at the most optimistic range predicted by the IPPC will accelerate wrack processing and beach metabolism, thus enhancing mineralization outputs, depleting the standing stock of inorganic nutrients on the upper beach, and thus increasing the release of nutrients toward the coastal environment and CO_2 into the atmosphere.

The result of this study can be extrapolated to different types of sedimentary shores where sandy sediment and macroalgal subsidies prevail, what it occurs in most of the coastal rims from temperate latitudes worldwide. Variables analysed were associated with the decomposition of algal tissues and degradative bacterial metabolism in the upper beach zone, what is not linked with features such as beach morphodynamics or beach size. Effect of different wrack subsidies (*e.g.* vascular plants from sea grass beds or salt marshes) or sediment granulometry (*e.g.* muddy or gravel beaches) deserve to be investigated in the near future.

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Conflict of interest

The authors whose names are listed in the Authors list certify that they have NO affiliations with, or involvement in, any organization or entity with any financial interest, or non-financial interest, in the subject matter or materials discussed in this manuscript. I confirm that the manuscript has been read and approved by all named authors. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Figure captions

Fig. 1 Average contents in (a) NH_4+, (b) NO_2-+NO_3- and (c) PO_4^{-3} in the sediment (μM g^{-1} of sand ± SE) for the warm-induced plots (OTC), and control plots of fresh *Saccorhiza*, aged *Saccorhiza* and bare sand over the 20 days of experiment. Within each variable, columns with the same letter indicate that the difference between the means was not statistically significant; columns with different letters indicate that they were significantly different.

Fig. 2 Increases in net contents in (a) NH_4+, (b) NO_2-+NO_3- and (c) PO_4^{-3} in the sediment (μM g^{-1} ± SE) for the warm-induced plots (black symbols) and control plots (open symbols) of fresh *Saccorhiza* (circles), aged *Saccorhiza* (squares) over the 20 days of experiment. Net contents was calculated through the gross values subtracted by the corresponding values in bare sand, either treatments or controls.

Fig. 3 Average CO_2 flux (μM m^{-2} s^{-1}) over the 20 days of experiment for the warm-induced plots (OTC) and control plots of fresh *Saccorhiza*, aged *Saccorhiza* and bare sand. Columns with the same letter indicate that the difference between the means was not statistically significant; columns with different letters indicate that they were significantly different.

Fig. 4 Total soil inorganic nitrogen vs. CO_2 flux for all the treatments and control plots; CO_2 values were standardized for the emission at the mean temperature along the daily cycle.

Fig. 5 Increases in net contents in DNA in the sediment (ng g^{-1}) for the warm-induced plots (squares) and control plots (circles) of fresh *Saccorhiza* (a) and aged *Saccorhiza* (b) over the 20 days of experiment. Net contents was calculated through the gross values subtracted by the corresponding values in bare sand, either treatments or controls.

Treatment	Mean Temperature	Mean daily Minimum Temperature	Mean daily Maximum Temperature	Degree-days
Fresh Algae control	22.17 (±3.76)	18.31 (±1.04)	27.68 (±3.55)	486
Fresh Algae OTC	22.20 (±2.10)	20.13 (±0.86)	25.01 (±2.46)	494
Aged Algae control	21.82 (±2.35)	19.27 (±1.03)	25.20 (±2.21)	487
Aged Algae OTC	22.16 (±2.34)	19.87 (±0.93)	25.45 (±2.55)	493
Bare Sand control	21.87 (±3.50)	18.07 (±1.35)	27.20 (±2.80)	484
Bare Sand OTC	22.00 (±2.60)	19.23 (±1.13)	25.86 (±2.97)	491

Table 1. Values of Temperature (ºC ± SD) along the experiment time for all the control and treatment plots. Data recorded by data loggers buried at 5 cm depth in the sediment. Degree days calculated as the area under the curve of the daily average temperature, according to Jaki & Wolfsegger (2009).